# Novel visualization studies of lignocellulosic oxidation chemistry by application of C-near edge X-ray absorption fine structure spectroscopy

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Received 15 July 2003; accepted in revised form 10 March 2004


*Key words:* Carboxylic acid functionalities, Hydrogen peroxide bleached pulp, X-ray absorption spectroscopy

**Abstract**

The research presented herein is the first attempt to probe the chemical nature of lignocellulosic samples by the application of carbon near edge X-ray absorption fine structure spectroscopy (C-NEXAFS). C-NEXAFS is a soft X-ray technique that principally provides selective interrogation of discrete atomic moieties using photoelectrons of variable energies. The X1A beam line of the National Synchrotron Light Source was employed for the specific purpose of observing carboxylic acid moieties that display a signature absorption band centered at 289 eV. This study caps a larger effort to support the mechanistic basis for lignocellulosic fiber chemical degradation induced by the disproportionation of hydrogen peroxide during fiber bleaching trials. It is shown that fibers that have been bleached with a hydrogen peroxide phase without removal of resident pendant metals (Mn, Cu, Fe) sustain significant macroscopic damage likely via classical Fenton-type radical reactions, as evidenced by a tensile reduction by over 30%. We present X-ray absorption spectra obtained using a scanning transmission X-ray microscope (STXM) at the end of a 2.5 GeV electron synchrotron that provided 1s $\rightarrow$ $\pi^*$ contrast-enhanced micrographs illustrating a random distribution of acid functionalities that were principally located on fiber surfaces. Control studies using non-bleached fibers demonstrated that very little signature carboxylic acid absorption patterns were present in the fibers, suggesting that these groups are an incriminating fingerprint for macroscopic fiber strength damage during non-radical suppressed bleaching trials.

## Introduction

The incorporation of hydrogen peroxide for the bleaching of lignocellulosics derived from wood pulping efforts represents a new paradigm for the production of environmentally compatible, low opacity lignocellulosics. Hydrogen peroxide is a relatively robust, extremely powerful oxidant whose oxidation capacity has been used in many arenas, particularly bleached pulp production, cotton scrubbing and bleaching, and wastewater treatment (Andreozzi et al. 2002; Gacen et al. 2002; Lopez et al. 2002). It is an established industrial process; yet, the mechanism of its

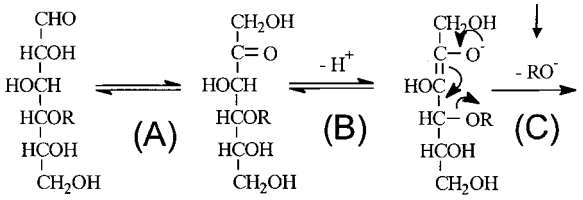
bleaching activity is not well elucidated for either pulp or textiles. Harnessing its oxidation potential is difficult, since several of its chemical disproportionation pathways tend to favor the production of non-selective radicals such as hydroxide in the presence of transition metals such as manganese or iron. Since cellulosics (including lignocellulosics) mainly comprise carbohydrate chains having glucose monomer repeat units up to $10^6$, any radical activity in the vicinity has the potential to induce $\beta$-elimination reactions resulting in chain cleavage that arise from highly efficient anomeric carbon oxidation. The insidious Fenton-type cycle has been shown repeatedly to induce severe cellulosic damage, especially in cotton fabrics, as a result of glycosidic cleavage reactions which are observed as severe attenuations in fiber tensile strength (Taher and Cates 1975). A typical series of reaction pathways leading to cellulosic damage is shown in Equations 1 and 2:

$$M^{2+} + H_2O_2 \rightarrow M^{3+} + \cdot OH + {}^-OH \quad (1)$$

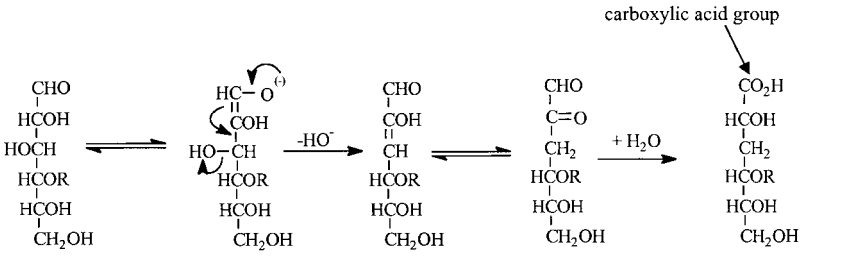
$M^{2+}$ = copper, iron, manganese ions.
(2) Peeling and stopping reactions:

Although the net result of these reactions is witnessed in the degeneration of the macroscopic properties, until this point no work has provided a rational, detailed assessment of the chemical pathways at the micro level that ultimately lead to the compromised physical properties. One of the main problems in ascertaining chemically induced damage at the microscopic level is the tremendous heterogeneity in the chemical composition of the pulp. Pulp is composed of a discrete array of biopolymers such as lignin, cellulose, and hemicelluloses. Traditional methods of visualization like AFM, SEM, TEM, and other microscopies cannot provide a detailed understanding of the chemical damage that is incurred in lignocellulosics through the disproportionation reactions of hydrogen peroxide. Recently it was determined that TOF-SIMS, however, can provide a remarkable topographical distribution of metal ions on lignocellulosics that is functionally dependent on bleaching and the arising chemistry (Mancosky and Lucia 2001a). Nevertheless, this method still suffers from an inability to probe chemical functional groups in a manner similar to NMR or other electromagnetic spectroscopic analytical

Loss of Carbohydrate Group




A = isomerization, B = enediol formation, C = β-alkoxy elimination (2)




glucometasaccharinic acid

("stopping product")

techniques. The measurement of chemical composition at high spatial resolution is a requisite for determining the changes occurring during hydrogen peroxide bleaching of pulp. Although such a measurement is challenging, high chemical specificity and resolution are possible employing near-edge X-ray absorption fine structure (NEXAFS) spectroscopy.

The high spatial resolution afforded by X-ray microscopy has the potential to characterize the sundry chemical changes occurring at the molecular level in pulp, a feat that has heretofore never been accomplished at the high level of accuracy and precision available to this technique. It is important to decipher the basis for the chemical changes in pulp, since these lignocellulosic samples are known to exhibit stress in their compositional integrity as a result of the oxidation reactions that occur during bleaching. For example, the intrinsic viscometric profile (flow) of the carbohydrate components of pulp is severely attenuated during extended bleaching conditions, a fact resulting from the cleavage and peeling reactions that reduce their degree of polymerization (oftentimes up to 50%) (Knill and Kennedy 2002). Additional macro level observables as a result of the decreases in polymerization are pronounced reductions in intrinsic fiber strength as measured by tensile and breaking length. Yet, there have been no clear correlations of the changes occurring at the molecular level with the macro level observables. Our initial efforts in the study of hydrogen peroxide-induced pulp damage suggested that carboxylic acid moieties are the chemical markers pursuant to the radical activity. We fortuitously discovered that the metal distribution of select metals (iron, manganese, magnesium) was systematically redistributed on a pulp surface following a bleaching event. They were distributed in concentrated aggregates or clusters in a manner suggesting the possibility of a severely oxidized site characterized by a preponderance of carboxylic acid functionalities. In fact, we had ESCA and NMR data that suggested a strong accretion of these groups, but were unable to definitively identify them.

In this study, we have therefore intended to focus on the chemical sensitivity and utility of the carbonyl core (C 1s, O 1s) $\rightarrow \pi^*_{C=O}$ transitions to probe the thesis of this work. The C 1s and O 1s NEXAFS spectra of a control and hydrogen peroxide bleached pulp sample are presented. C-NEXAFS has been used in the past to probe the electronic structure of organic carbon within microheterogeneous samples similar to ours to provide very conclusive functional group information (Cody et al. 1995, 1996). The present work describes our efforts to clearly establish the localization of carboxylic acid functionalities in a distribution and topology that is concurrent with their development as a result of the oxidative damage process incurred by pulp fibers.

## Experimental

### *Preparation of substrates*

All samples used were obtained from a Northern black spruce tree approximately 30 year of age that was sectioned according to age (juvenile wood – top 8 ft, mature wood, bottom trunk of tree), debarked, chipped, and stored. All pulp samples were prepared according to standard kraft pulping techniques by an independent laboratory and in-house.[1] The control sample (unbleached) was a typical mature hand sheet sample, whereas the bleached sample was bleached according to protocols described previously (Mancosky et al. 2001b). The samples were cut into square sheets of approximately 2 cm × 2 cm size. The samples were then prepared for mounting onto a standard TEM gold grid surface using a modified epoxy resin embedding procedure. The following procedure was carried out to ensure proper epoxy-fiber embedding and penetration to avoid delamination effects during microtoming: the pulp samples were soaked piecemeal in water, ethanol (three times), and propylene oxide (three times) for 10 min each. Finally, they were soaked for curing in propylene oxide/Epon resin (1:1 w:w) followed by neat Epon for 1 h each to ensure homogeneous distribution of Epon by propylene oxide. The sample was then placed under vacuum overnight to remove excess propylene oxide and sandwiched between polyethylene sheets. After curing of the Epon resin, the samples were precisely microtomed according to standard procedures, mounted onto TEM grids,

[1]We acknowledge Roberta J. Hanna of SAPPI, formerly Potlatch, and Gopal Goyal, formerly of Potlatch/SAPPI, now at International Paper, for their expert pulping work.

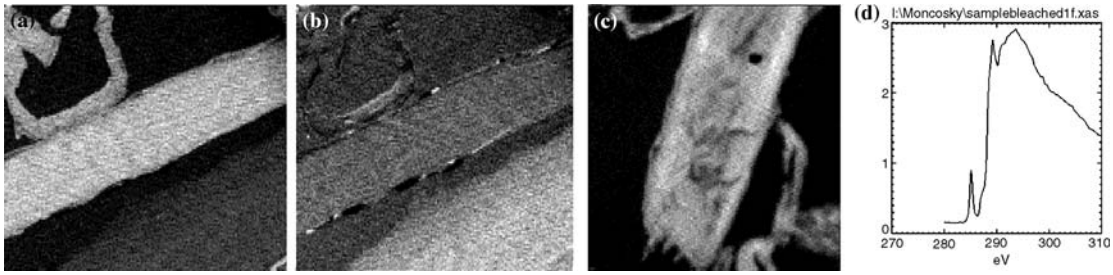



*Figure 1.* Photomicrographs which are illustrative of the following: (a) unbleached fiber (white) in resin (gray) 72 $\mu$m across; (b) the same photomicrograph as (a) except with contrast adjusted to highlight pits; (c) a bleached fiber section approximately 50 $\mu$m across; and (d) a sample steady-state absorbance spectrum of the fiber in (c).

and stored for transport to Brookhaven National Laboratories.[2]

*Carbon micro-NEXAFS measurements*

Carbon NEXAFS data were acquired using the scanning transmission X-ray microscope (STXFM) and microspectrometer located at the X1A beam line that are located at the National Synchrotron Light Source at Brookhaven National Laboratories in Upton, NY. The X-ray source is an undulator on the 2.5 GeV electron storage ring. The current configuration of the STXFM monochromator is capable of 0.3 eV energy resolution and a spatial resolution of approximately 40 nm. X-ray microfocusing is done by diffraction limited optics using a Fresnel phase-zone plate objective and an order sorting aperture (Jacobsen et al. 1991). In order to obtain microspectra, the monochromator must be in the scanning mode while the sample is simultaneously stepped along the $z$-axis to maintain focus (a stepper motor drive is used with drift up to 1 $\mu$m in the $x$–$y$ axes). The actual window for analysis is approximately $100 \times 100$ nm due to these positioning errors (see Cody et al. 1998). The data collection was accomplished by acquiring an absorption spectrum spanning the energy range of 280–310 eV which was corrected for background noise/storage current drift by subtraction of a spectrum without the sample. The data are presented in the absorption mode as $-\log(T/T_0)$, in which $T$ and $T_0$ are the transmittance of the sample and the background, respectively. Typical spectra span a wavelength range of 3 Å and include 512 points with a dwell time of 100 ms. Energy calibration is provided by comparison of the position of the 1s $\rightarrow$ $\pi^*$ transition with the Rydberg transitions of $CO_2$ that is bled into the irradiated volume (Ma et al. 1997).

[2]Personal correspondence with Professor H. Nanko (IPST).

## Results and discussion

One of the first challenges we encountered in our early work was differentiating the signal that evolved from the embedding matrix versus the paper sample given the absorption maxima for aromatic residues at the near C edge. We found that clear contrast at 285 eV was nevertheless possible for the hydrogen peroxide bleached black spruce fiber sections, as shown in Figure 1a–d in which the fiber and the background can easily be distinguished.

Once differentiation of the matrix and sample components was established, we initiated a stacked spectral acquisition to compare and contrast the various regions in a 45 $\mu m^2$ area. It was found from our analysis that there was a relatively pronounced spectrum after employing the 289 eV energy for visualization which we were able to correlate to the COOH 1s $\rightarrow$ $\pi^*$ transition. Figure 2 demonstrates a representative spectral output at this energy.

It is easy to observe the COOH 1s $\rightarrow$ $\pi^*$ absorption that is localized along the edge of the fiber sections, since it is depicted as a more intensely concentrated dark area. Interestingly, the spectral window for this transition was observed to be relatively narrow. STXM is a transmission technique and therefore dark bands are occasionally visible due to folds in the sample, effectively doubling the thickness and not allowing for the transmission of the X-ray energy at any wavelength. This was tested by sweeping multiple

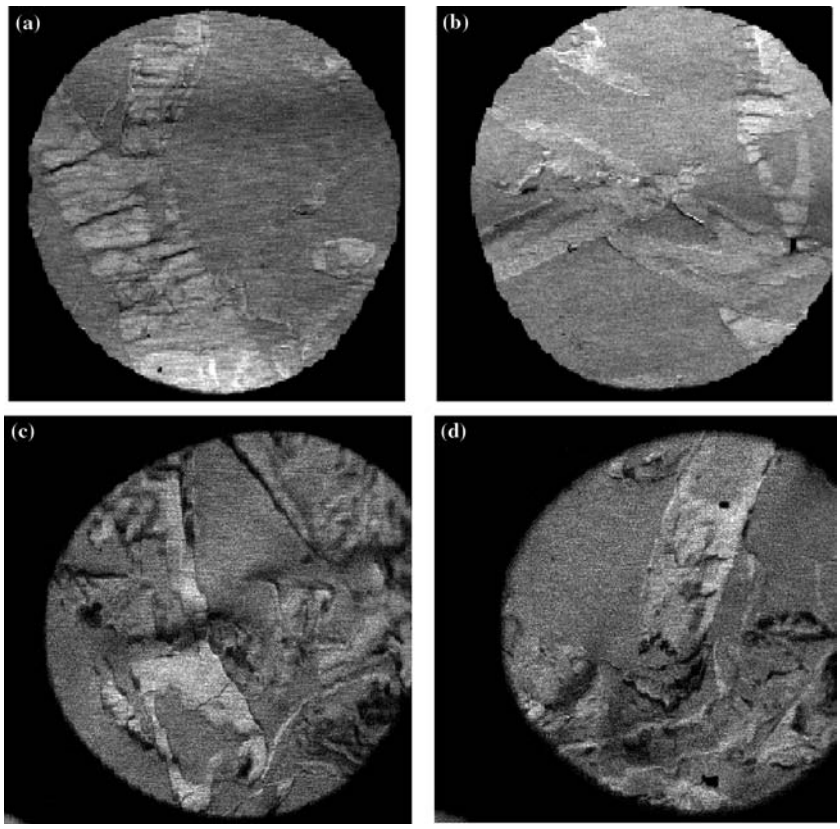



*Figure 2.* Photomicrographs representing the following: (a, b) unbleached fibers at 289 eV; (c, d) bleached fibers at 289 eV (all holes are approximately 125 $\mu$m across).

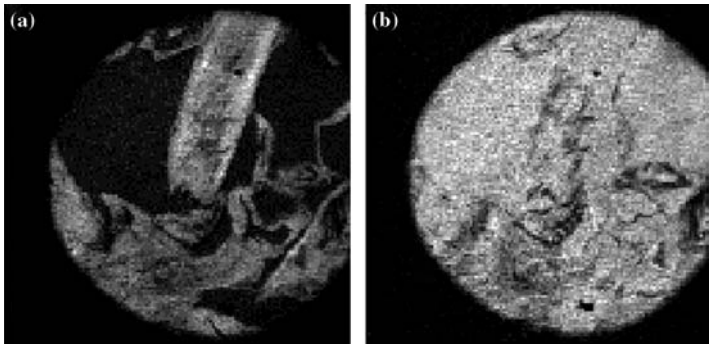



*Figure 3.* (a) Absorbance profile of bleached fibers at 288.5 eV; (b) absorbance of bleached fibers at 289.5 eV (all holes are approximately 125 $\mu$m across).

wavelengths and was found not to be the case for any of the carboxylic rich regions. Figure 3 demonstrates the spectral display for the same fiber using 0.5 eV increments on either side of 289 eV. It is obvious that 289 eV is indeed the maximum for the COOH transition. Although work done by Cody indicates that this transition is at lower energies (287.5 eV), it is well appreciated that the electronic environment plays a critical role in affecting C 1s transition energies. The analysis of the maturation of the vitrinite coals as studied by Cody provides an approximate value for the near edge transition that is clearly a function of its interaction with similar energy transitions.

Our previous studies strongly suggested that the oxidation chemistry that is observed in the hydrogen peroxide bleaching of pulp fibers mainly occurs at the surface of the fiber.[3] We were able to develop a working theory through the data collected from potentiometric, TOF-SIMS, ESCA, and SEM measurements that indicated that the chemical differences contributing to fiber tensile strength degradation mainly arise from radical chemistry that gives rise to oxidation of the carbohydrates and lignin, ultimately providing carboxylic acid functionalities. In order to lend sufficient evidence to this theory, it was necessary to acquire a large sampling of fibers to observe the localized topographic occurrence of these acids that display a distinct spectral signature at 289 eV. As evidenced in the previous figures, the bleached samples all display a degree of absorption at 289 eV that is mainly localized on the periphery of the substrate. This localization is consistent with a surface-mediated oxidation chemistry that arises from metal-catalyzed disproportionation of the hydrogen peroxide. Control studies in our laboratories as well as those of others indicate that this event can be mitigated by efficient removal of the metals through appropriate chelation measures (Líden and Ohman 1997). In fact, these topological signatures are consistent with the surface acid enrichment of hydrogen peroxide-bleached pulp data acquired in our laboratories. The striking absence of these motifs on the periphery of the control pulp fibers provides ample support for the proposal that acid accretion is a by-product of hydrogen peroxide-mediated pulp damage, mainly incurred in the carbohydrate moieties, specifically cellulose. The totality of our initial data suggested that the brunt of the damage sustained by kraft fibers was borne by the load-bearing portion or cellulose regions. Interestingly, supporting data in this laboratory has shown that oxygen-mediated bleaching schemes cause an enrichment of crystalline cellulose domains with a concomitant drop in the level of amorphous cellulose domains (Fu and Lucia 2004).

The current work represents the first concerted and definitive effort at establishing the nature of the fiber damage sites that occurs during the hydrogen peroxide bleaching of non-chelated fibers. This technique is extremely powerful for the determination of organic functional groups with high specificity and without the issue of spectral noise arising from coupled transitions, since the analytical method is essentially a quasi-atomic absorption technique.

[3]Work done under the auspices of an industrially funded internal consortium at IPST strongly suggested this chemistry. The work is currently in press in J. Colloid Interface Sci. (2004).

## Conclusions

The current preliminary data provides the first known X-ray interrogation of the salient chemical features of hydrogen peroxide bleached kraft softwood pulps that define the molecular origin of the damage that the fibers sustain during the bleaching process. The carboxylic acid moieties that absorb strongly at 289 eV are present in abundance at the edges of the macerated fibers, lending significant evidence to the postulation that hydrogen peroxide-induced fiber damage is a topochemical Fenton event that occurs extensively in the presence of metals. These superficial metals have been extensively authenticated by TOF-SIMS methods as well as standard bulk ICP. This classical study promises to bear much fruit for elucidating the chemistry that is responsible for propagating and/or terminating radical-initiated events.

## Acknowledgements

Research was carried out (in whole or in part) at the National Synchrotron Light Source, Brookhaven National Laboratory, which is supported by the US Department of Energy, Division of Materials Sciences and Division of Chemical Sciences, under Contract No. DE-AC02-98CH10886. Data were taken using the X-1A STXM developed by the group of Janos Kirz and Chris Jacobsen at SUNY Stony Brook with support from the Office of Biological and Environmental Research, US DOE under contract DE-FG02-89ER60858, and the NSF under Grant DBI-9605045. The zone plates were developed by Steve Spector and Chris Jacobsen of Stony Brook and Don Tennant of Lucent Technologies Bell Labs, with support from the NSF under grant ECS-9510499. We would also like to thank IPST at the Georgia Institute of Technology and its member companies for their support.

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